Extremophiles (2017) 21:259–269
DOI 10.1007/s00792-016-0895-x

ORIGINAL PAPER

Antarctomyces pellizariae sp. nov., a new, endemic, blue, snow resident psychrophilic ascomycete fungus from Antarctica

Graciéle C. A. de Menezes[1] · Valéria M. Godinho[1] · Bárbara A. Porto[1] · Vívian N. Gonçalves[1] · Luiz H. Rosa[1]

Received: 1 September 2016 / Accepted: 18 November 2016 / Published online: 30 November 2016

Abstract In the present study, we have identified and characterised a new snow resident ascomycete blue stain fungus from Antarctica named *Antarctomyces pellizariae* sp. nov. Menezes, Godinho, Porto, Gonçalves and Rosa, using polyphasic taxonomy techniques. This fungal species was recovered from the seasonal snow of the Antarctic Peninsula. *Antarctomyces pellizariae* displayed different macro- and micromorphology when compared with *A. psychrotrophicus* Stchigel and Guarro, the only other *Antarctomyces* species reported until date. *Antarctomyces pellizariae* showed psychrophilic behavior and very low growth rate at 22–25 °C, quite different from *A. psychrotrophicus* that has a higher growth rate at mesophilic temperatures. In addition, micromorphological characteristics and the analysis of the nuclear rDNA internal transcribed spacer, β-tubulin, and RNA polymerase II regions revealed that *A. pellizariae* is a new species that is related to *A. psychrotrophicus* and *Thelebolus* species. Since the Antarctic Peninsula is reported to be one of the main regions of the earth experiencing the effects of global change in climate, species, such as *A. pellizariae,* might provide information about these effects on the endemic Antarctic biota. In addition, *A. pellizariae* displayed psychrophilic behavior and might be a source of interesting anti-freeze compounds that might prove useful in biotechnological processes.

Keywords Antarctica · *Antarctomyces* · Fungi · New species · Snow

Introduction

Antarctica is the largest continent on the planet, with an extreme climate. This makes it a unique field laboratory for taxonomy, diversity, ecology, evolution, and biotechnological studies on polar fungi. Fungi represent an important part of the microbiota of Antarctica. Important representatives of the polar fungi community are the filamentous fungi consisting of species in the phyla Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota, and Basidiomycota (Ruisi et al. 2007). In Antarctica, fungi have been reported in different substrates, such as soil, rocks, woody components, freshwater, macroalgae, and plants (Ellis-Evans 1985; Tosi et al. 2002; Arenz et al. 2006; Rosa et al. 2009; Loque et al. 2010; Bridge and Spooner 2012).

Among the different substrates that shelter microbial communities in Antarctica, snow represents an interesting and unknown habitat for bacteria, archaea, and eukarya. Approximately 35% of the land surface is covered by snow (Miteva 2008; Margesin and Miteva 2010). These resident microbial communities might have an active influence on the nutrient dynamics and hydrochemistry of snow (Larose et al. 2013). According to Margesin and Miteva (2010), the snow habitat represents the constant aeolian fluxes of dust, microorganisms, and other biological material deposited with precipitation. According to Miteva (2008), snow habitats are characterised by permanent low temperatures, low nutrient availability, and in high altitudes and polar regions,

Communicated by A. Oren.

Electronic supplementary material The online version of this article (doi:10.1007/s00792-016-0895-x) contains supplementary material, which is available to authorized users.

✉ Luiz H. Rosa
lhrosa@icb.ufmg.br

[1] Department of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, C. P. 486, Belo Horizonte, MG CEP 31270-901, Brazil

and high levels of UV radiation. Few studies have characterised the microbial communities in the snow of Antarctica, with most of them characterising bacteria and microalgae (González-Toril et al. 2008; Fujii et al. 2010; Lopatina et al. 2013); only Fujii et al. (2010) described the yeasts found in the red snow in Antarctica.

In the Antarctic summer of 2015, we collected snow samples from different Antarctic islands to isolate its fungal communities. Among these fungal communities, we discovered a filamentous fungus with deep blue-coloured colonies on Sabouraud agar, first identified as *Antarctomyces* sp. UFMGCB 12416 by preliminary molecular taxonomy. The detailed polyphasic analysis showed strong evidence that *Antarctomyces* UFMGCB 12416 represented a new ascomycete species.

Methods

Snow sampling, concentration, and fungus isolation

The snow sample was collected in the Coppermine Peninsula (62°37′941″S; 59°70′400″W), Robert Island, in the South Shetland Islands, Antarctica in the austral summer season in the December of 2015. Approximately 10 kg of snow from the uppermost 1.5 m layer was collected using a sterile plastic scoop and stored in sterilised plastic bags of 30 L capacity. Sterilised suits and gloves were worn during sample collection to minimise contamination during sampling. The snow was melted in the microbiology laboratory on board the Brazilian polar ship admiral Maximiano over a period of about 12 h with occasional mixing. This process resulted in 10 L of water. A total of 1.5 L of melted snow was filtered through a 0.45 µm membrane with 47 mm diameter (Millipore, USA) in triplicate. The membranes were placed on Sabouraud agar (Acumedia, India) containing 200 µg mL^{-1} of chloramphenicol (Sigma, USA) and incubated at 10 °C for 30 days. The fungus was purified in new Petri dishes containing Sabouraud agar and deposited in the Collection of Microorganisms and Cells of the Universidade Federal de Minas Gerais, Brazil, under the code UFMGCB 12416 in cryotubes at −80 °C and in distillate-sterilised water (Castellani 1967) at room temperature, which are available to be accessed by other scientists.

Macro- and micromorphological characterization

Fungal macroscopic parameters (colony colour, texture, reverse colour, border type, and radial growth rate) and colony diameters were observed on different media, as described in the following. Colours follow the specification proposed by Kornerup and Wanscher (1984). The *Q* values were obtained by length/width of individual ascospores, conidia, and chlamydospores. *Antarctomyces* sp. UFMGCB 12416 was inoculated into three point cultures on the following media: potato carrot agar [PCA, potatoes 20 g, carrot 20 g, agar agar 20 g (Kasvi, Brazil), and sterilised distilled water 1 L], oatmeal agar [OA; oat flakes 30 g, agar agar 15 g (Kasvi, Brazil), and sterilised distilled water 1 L], potato dextrose agar (PDA; Merck, Germany), malt extract agar [MEA; glucose 20 g (Synth, Brazil), malt extract 15 g (Merck, Germany), peptone 1 g (Kasvi, Brazil), and agar 20 g (Kasvi, Brazil)], and Sabouraud agar. All media were incubated for 7 and 14 days at 4, 10, 15, and 25 ± 2 °C. Media under the same conditions were used to determine the microscopic parameters (hyphae, conidiophores, and conidia) using slide cultures mounted in lactophenol and viewed under a microscope (Leica DM750, Germany).

For scanning electron microscopy, the fungus *Antarctomyces* sp. UFMGCB 12416 was inoculated on PCA, PDA, and Sabouraud agar media and incubated at 10 and 25 °C for 14 days. The mycelia were fixed in 2% glutaraldehyde in 0.1 M $NaPO_4$ buffer and washed in buffered 1% OsO_4 for 2 h. The material was dehydrated using a graded ethanol series (10, 25, 40, 60, 75, 85, 95, and 100%) for 15 min per concentration. The material was dried in a critical point drying apparatus, sputter-coated with gold, and viewed with an FEI Quanta 200 SEM, USA.

Fungal growth responses to different temperatures

Plugs of 4 mm^2 from 7-day-old pre-cultures of *Antarctomyces* sp. UFMGCB 12416, grown at 10 °C on PDA, were inoculated onto Petri dishes containing PDA medium and incubated at 5, 10, 15, 20, and 25 ± 2 °C. Plates were incubated in triplicate, and after 7 and 14 days, the colony diameters were measured in mm.

Molecular identification

DNA was extracted as per the protocol described previously in Rosa et al. (2009). The internal transcribed spacer (ITS) region was amplified with universal primers ITS1 and ITS4 (White et al. 1990). Amplification of the ITS region was performed, as described by Rosa et al. (2009). In addition, amplification of the β-tubulin (Glass and Donaldson 1995) and ribosomal RNA polymerase II genes (RPB2) (Houbraken et al. 2012), which are commonly utilised to identify fungal species of the same genera with low intraspecific variation was done with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively. Representative consensus sequences of *A. pellizariae* were deposited into GenBank (KX576510, KX790790, and KY100007), TreeBASE (19625), and Mycobank (MB 817786). To achieve species-rank identification based on ITS, β-tubulin, and RNA polymerase II, the consensus

sequence was aligned with sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul et al. Altschul et al. 1997). Sequences of *A. pellizariae* were compared with different sequences of type species as well as the ITS sequence of the ex-type species *Antarctomyces psychrotrophicus* (AJ133431), both deposited in the GenBank database with estimations conducted using MEGA Version 6.0 (Tamura et al. 2013). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1000 replicate runs. The information about fungal classification generally followed the dictionary of Kirk et al. (2008), MycoBank (http://www.mycobank.org), and the Index Fungorum (http://www.indexfungorum.org).

Results

Preliminary taxonomic analysis

Melting snow from Antarctica revealed a fungus that was labelled UFMGCB 12416. It displayed an unusual deep blue colour on Sabouraud agar at 10 °C (Suppl. Fig. 1), which drew our attention, as it could possibly be a new taxon. Preliminary BLAST-based comparison of the ITS sequences of the UFMGCB 12416 taxon with the GenBank sequences showed that the three closest species were fungal endophyte (HQ335307) recovered from the moss *Polytrichum strictum* at Barton Peninsula, Antarctica (query coverage = 99%, identity = 100%); uncultured fungus (KC965762) isolated from the soil of Happy Valley, Alaska, USA (query coverage = 100%, identity = 99%); and *Antarctomyces psychrotrophicus* (GU004189) obtained from the soil from an undescribed region in Antarctica (query coverage = 98%, identity = 100%).

According to Stchigel et al. (2001), *Antarctomyces* possess peculiar morphological features that make the genus easy to identify. These characteristics include the following: mainly submerged mycelium, composed of septate, branched and unbranched, anastomosing, hyaline hyphae; ascomata composed of naked asci, without excipulum; asci that are ellipsoidal to subglobose, unitunicate, non-catenate, and eight-spored; absent paraphyses; ascospores that are ellipsoidal to fusiform, hyaline, spinulose, without germ pores, and one-celled.

Macro- and micromorphological analyses

After the detection of the preliminary genetic and morphological characteristics, we identified the fungus UFMGCB 12416 as an *Antarctomyces* species. However, the fungus UFMGCB 12416 when cultured on PDA at 22–25 °C displayed slower growth, different from that of *A. psychrotrophicus*. The mycelial characteristic suggested that the fungus UFMGCB 12416 could represent a new species, and its detailed molecular and morphological characteristics were determined. The macro- and micromorphological characteristics of *A. pellizariae* on five different media (Figs. 1, 2) are described below.

PCA after 14 days

At 11–12 °C

The colonies were 26–27 mm in diameter, and plane, thin, zonate, vegetative mycelium was mainly submerged and uncoloured, reverse uncoloured. Ascomatal initials began to develop from the coiling of two side branches, occasionally disposed in tandem. Ascomata composed of naked asci were seen, singly or in groups of six, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were scarce, 17–23 × 8–17 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thickwalled, non-catenate, eight-spored, and developed form croziers. Ascospores were 6.5–9 × 4.5–6 µm in size (Q = 1.4–1.5), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines *c*. 0.3–0.5 µm long. Anamorph stage: conidia and chlamydospores were absent.

At 22–25 °C

The colonies were 16.5–18 mm in diameter, plane, thin, white with yellowish centre, with irregular margins, anastomosing, reverse uncoloured, with septate hyphae; hyphae 1.5–2 µm broad, thin to thick-walled. Ascomatal initials were scarce and began developing from the coiling of the two side branches, occasionally disposed in tandem. Ascomata were scarce in number, composed of naked asci, single, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 8.5–20 × 6–13 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 5–8.5 × 3–6 µm in size (Q = 1.7–1.4), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, one-celled; spines *c*. 0.3–0.5 µm long. Anamorph stage: conidia were absent. Chlamydospores were abundant, 3–5 × 5–10 µm in size (Q = 0.6–0.5), irregular, single or forming long chains, and one or two-celled.

PDA after 14 days

At 11–12 °C

The colonies were 15–16.5 mm in diameter at 11–12 °C, rough, with long radial furrows, irregular margins,

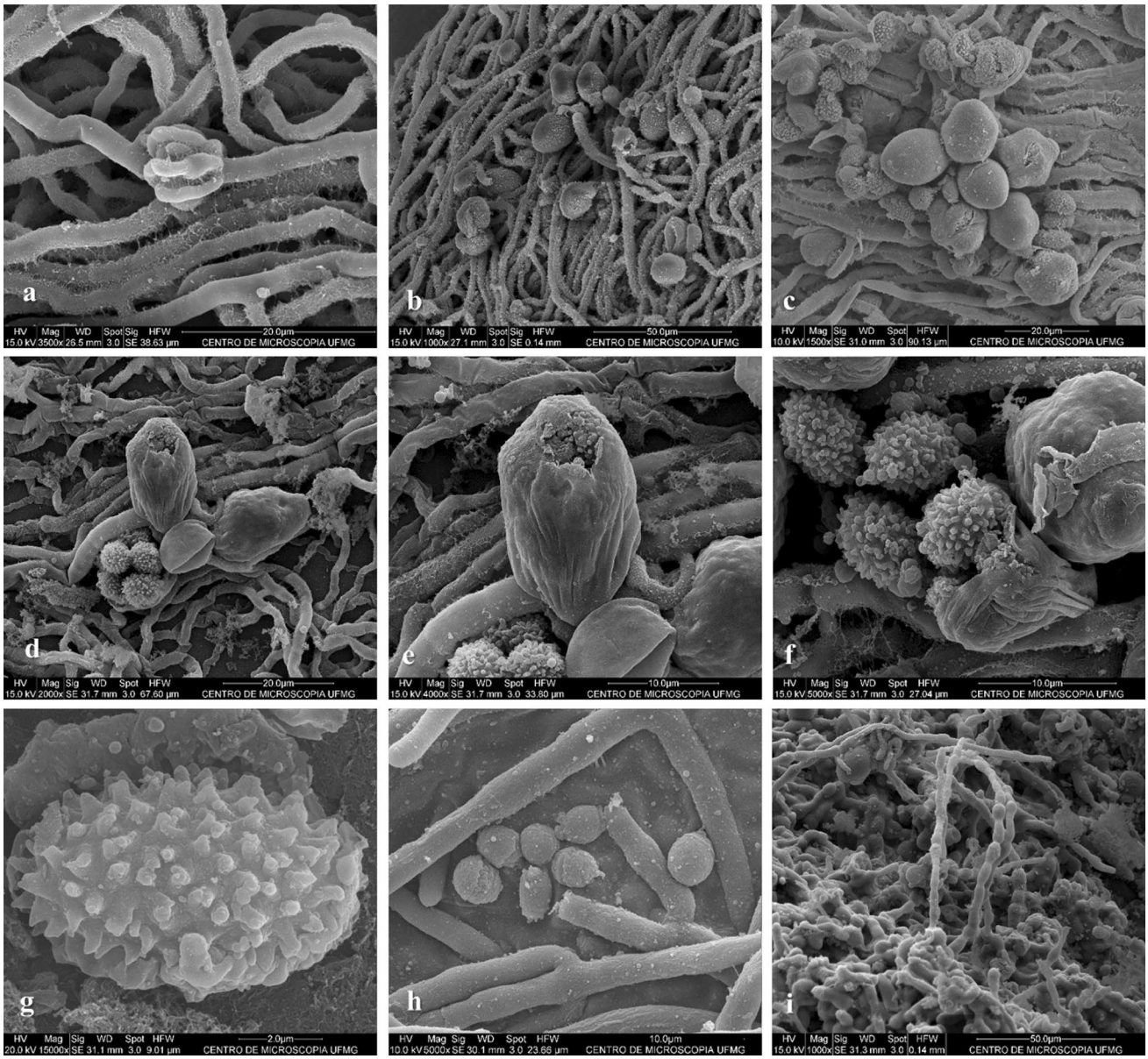


Fig. 1 Optical micrographs of *Antarctomyces pellizariae* after 14 days of growth. **a** Asci on PCA at 10–12 °C. **b** Free ascospores with spinulose surface and the thick wall on PCA at 10–12 °C. **c** Cluster of conidia on PDA at 22–25 °C. **d** Anastomosis and chlamydopores on PCA at 22–25 °C. **e** Ascomata initials on PCA at 22–25 °C. **f** Ascomata initials on OA at 10–12 °C. **g** Asci with ascospores on OA at 10–12 °C. **h** View of septate hypha and ascomata initial on MEA at 4–6 °C. **i** Asci and ascospores on MEA at 10–12 °C. **j** Asci and ascospores on Sb at 10–12 °C. *PCA* potato carrot agar, *PDA* potato dextrose agar, *OA* oat agar, *MEA* malt extract agar, *Sb* Sabouraud agar

vegetative mycelium mainly submerged, dull blue (M 23D4), light blue funicles in the central area, composed of sterile hyphae, anastomosing, reverse with the same colour. Abundant ascomatal initials began to develop from the coiling of two side branches. Ascomata composed of naked asci, single or in groups of two, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 6–11 × 10–19 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 3–5 × 4–7 µm in size (Q = 0.8–0.7), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines c. 0.3–0.5 µm long. Anamorph stage: conidia were 6.5–7.5 × 4–5 µm in size (Q = 1.6–1.5), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were 9–11 × 6–7 µm in size

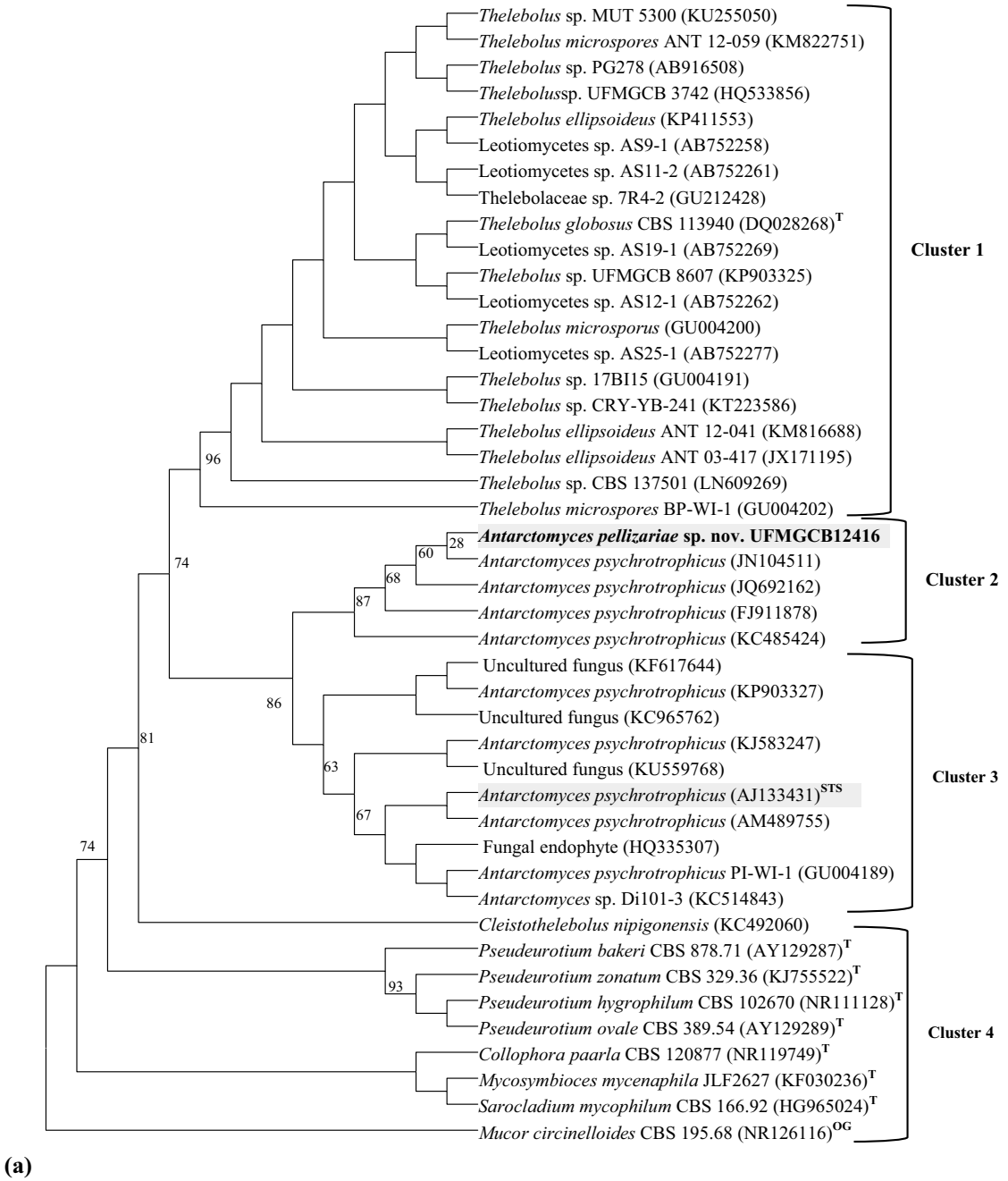
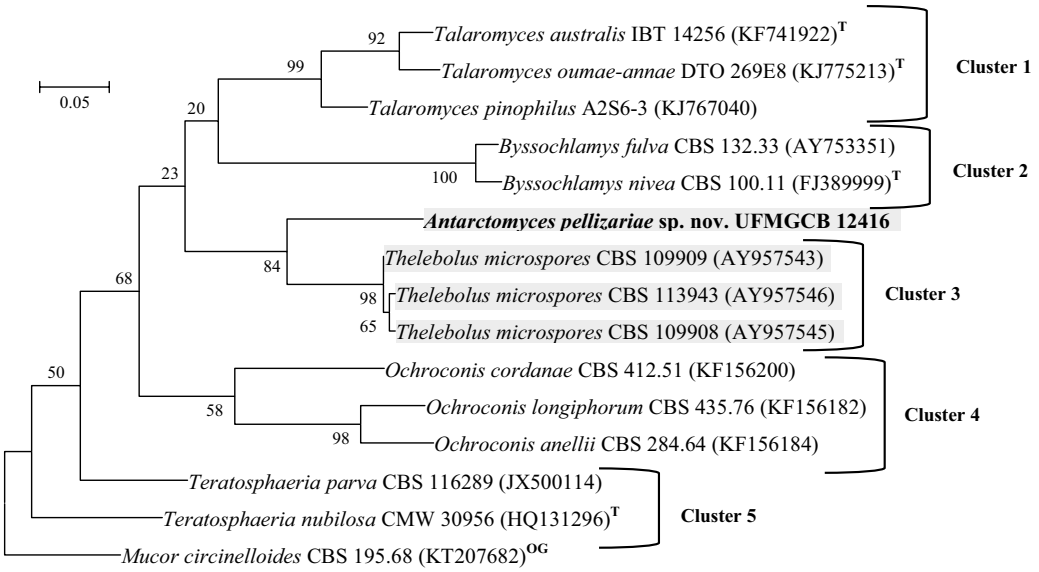


Fig. 2 Scanning electron micrographs of *Antarctomyces pellizariae* on potato carrot agar (PCA) at 10–12 °C after 14 days. **a** Ascomata initials. **b** Young asci. **c** Cluster of young asci. **d** Mature asci and release ascopores. **e** Mature asci. **f** Ascopores releasing of the asci. **g** Free ascospores with spinulose surface and the thick wall. **h** Different conidia. **i** Chlamydospores

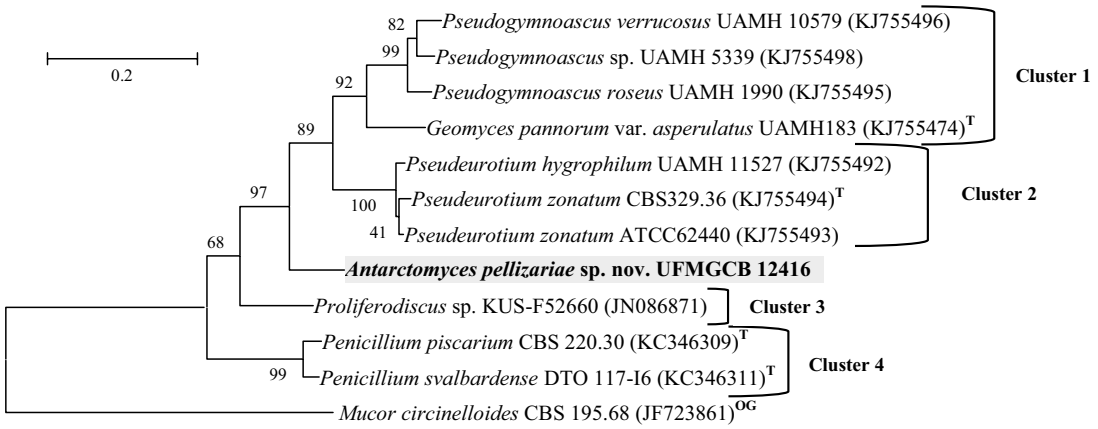
(Q = 1.5–1.6), irregular, single or forming long chains, and one or two-celled.

At 22–25 °C

The colonies were 6–7 mm in diameter after 14 days, rough, margins irregular, vegetative mycelium mainly submerged, yellowish, reverse yellowish. Asci were absent. Anamorph stage: conidia were 4.5–9 × 4–5 µm in size (Q = 1.1–1.8), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were abundant, 7–14 × 6–11 µm in size (Q = 1.2–1.3), irregular, single or forming long chains, and one or two-celled.



(b)



(c)

Fig. 2 continued

OA after 14 days

At 11–12 °C

The colonies were 20–21 mm in diameter at 11–12 °C, plane and thin; vegetative mycelium mainly submerged, uncoloured, reverse uncoloured. Ascomata were composed of naked asci, single or in groups of 1–4, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci measured 10–23 × 10–16.5 µm in size, were subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 7–8 × 3–4 µm in size (Q = 2.3–2), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines with 0.3–0.5 µm long. In the anamorph stage, conidia were absent. Chlamydospores were scarce and measured 3–6 × 3–5 µm in size (Q = 1–1.2), were irregular, single, or forming long chains, and were one or two-celled.

At 22–25 °C

The colonies were 10–21 mm in diameter, plane, thin, fimbriate margins, vegetative mycelium mainly submerged, uncoloured, reverse uncoloured, anastomosing. Ascomatal initials, scarce in number, began to develop from

the coiling of two side branches, occasionally disposed in tandem. Asci were absent. Anamorph stage: conidia were 5–9 × 3–4 µm in size (Q = 1.7–2.6), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were very abundant in long chains, 6–11 × 5–7 in size (Q = 1–1.6), irregular, single or forming long chains, and one or two-celled.

MEA after 14 days

At 10–12 °C

The colonies were 55–59 mm in diameter at 10–12 °C and 30–32.5 mm in diameter at 4–6 °C, plane, with the vegetative mycelium mainly submerged, yellowish, reverse yellowish, anastomosing. Moniliform mycelium and asci were present. Abundant ascomatal initials began to develop from the coiling of two side branches. Ascomata were composed of naked asci, single or in groups of 2–4, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci measured 16–22 × 9–14 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 5–7 × 3–4 µm in size (Q = 1.7–1.8), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines with 0.3–0.5 µm long. Anamorph stage: conidia absent. Chlamydospores were 5–11 × 5–7 µm in size (Q = 1–1.6), irregular, single or forming long chains, and one or two-celled.

At 22–25 °C

The colonies were 8 mm in diameter, with the same culture characteristics as in OA. Moniliform mycelium was present. Asci, chlamydospores, and conidia were absent.

Sabouraud agar after 14 days

At 10–12 °C

The colonies were 26–27 mm in diameter, cerebriform, front and reverse white to soft blue. Ascomatal initials began to develop from the coiling of two side branches, occasionally disposed in tandem. Ascomata were composed of naked asci, single or in groups of 2–5, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 18.5–20 × 13–15 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed form croziers. Ascospores were 13–18.5 × 15–20 µm in size (Q = 0.85–0.9), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines c. 0.3–0.5 µm long. Anamorph stage: conidia and chlamydospores were absent.

At 22–25 °C

The colonies were 26–27 mm in diameter, irregular, vegetative mycelium mainly submerged, front reverse yellowish, mycelium mainly submerged, composed of hyaline, branched and unbranched, anastomosing, with septate hyphae; hyphae 2–4 µm broad, thin to thick-walled. Ascomatal initials began to develop from the coiling of two side branches, occasionally disposed in tandem. Ascomata were composed of naked asci, single, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 11.5–14 × 6.5–7 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed form croziers. Ascospores were 4.5–8.5 × 3–6 µm in size (Q = 1.5–1.4), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, and one-celled; spines c. 0.3–0.5 µm long. Anamorph stage: conidia were 7–9 × 4 µm in size (Q = 1.75–2), subglobose to irregularly cylindrical, hyaline, smooth, thick-walled, aggregated in slimy masses, and one-celled. Chlamydospores were abundant, 7–11 × 4.5–7 µm in size (Q = 1.5–1.6), irregular, single or forming long chains, and one or two-celled. Sporothrix-like anamorph was present.

In summary, the morphology of *A. pellizariae* is different when compared with that of *A. psychrotrophicus* (Table 1). The colonies growing on all the media (mainly on MEA) at 22–25 °C were 8 mm in diameter in the case of *A. pellizariae* where as the same for *A. psychrotrophicus* was 54–58 mm. The two species also differed with respect to the number of asci groups, asci, ascospores, chlamydospores measurements, and absence of conidia. The colour of the colonies of *A. pellizariae* on PDA, OA, and MEA are different compared with that of *A. psychrotrophicus*. In addition, *A. pellizariae* showed abundant asci on MEA at 10–12 °C in contrast with *A. psychrotrophicus*, which, according to Stchigel et al. (2001), does not produce any asci.

We also evaluated the mycelial growth rate of *A. pellizariae* at different temperatures for 7 and 14 days (Suppl. Fig. 3). At 14 day, *A. pellizariae* displayed the most growth at 15 and 20 ± 2 °C (colonies diameters of 43.5 ± 4 and 57 ± 5.5 mm, respectively), followed by 10 and 4 ± 2 °C (33.5 ± 4 and 26 ± 5.5 mm diameter, respectively). In addition, *A. pellizariae* showed the slowest growth at 25 ± 2 °C (11.5 ± 0.5 mm diameter), indicating its psychrophilic behavior.

Table 1 Main macro- and micromorphological differences between *Antarctomyces pellizariae* and *Antarctomyces psychrotrophicus*

	Colour and measurement (in mm) of the colonies on different media at 22–25 °C					Micromorphology characteristics (measurement in µm) on PCA at 22–25 °C				
	PCA	PDA	OA	MEA	Sb	Asci number	Asci	Ascospores	Chlamydospores	Conidia
Antarctomyces pellizariae	Yellowish 16.5–18	Yellowish 6–7	Uncoloured 10–21	Uncoloured 8	Yellowish 26–27	Single	8.5–20 × 6–13	5–8 × 3–6 (Q = 1.7–1.3)	3–5 × 5–10 (Q = 0.6–0.5)	Absent
Antarctomyces psychrotrophicus	White 33–47	Uncoloured 65–71	Uncoloured 50–55	Uncoloured 54–58	nd	Single or groups of 2–7	15–19 × 12–13	7–10 × 4–5.5 (Q = 1.7–1.8)	10–15 × 5–8 (Q = 2–1.9)	3–20 × 2–5 (Q = 1.5–4)

Characteristics of the *Antarctomyces psychrotrophicus* are according to Stchigel et al. (2001)

PCA potato carrot agar, *PDA* potato dextrose agar, *OA* oat agar, *MEA* malt extract agar, *Sb* Sabouraud agar, *nd* not determined

Phylogenetic analysis

To prove our hypothesis and to clarify the taxonomic position of the new *Antarctomyces* species, we performed a phylogenetic study based on the sequences of the ITS, β-tubulin, and RNA polymerase II regions (Fig. 3). Detailed phylogenetic analysis of the ITS region of the sequences of *A. pellizariae* with the nearest taxa obtained from GenBank (Fig. 3a) showed that *A. pellizariae* forms a distinct cluster (cluster 2) similar to four *Antarctomyces* taxa (JN104511, JQ692162, FJ911878, and KC485424), which might also be *A. pellizariae* taxa, but different from cluster 3, which includes the type species *A. psychrotrophicus* (AJ133431). In addition, the *A. pellizariae* cluster showed phylogenetic differences when compared with *Thelebolus* (a sister species) and Leotiomycetes and *Pseudeurotium*, *Collophora*, *Mycosymbioces,* and *Sarocladium* taxa. In addition, the ITS sequence of *A. pellizariae* displayed 11 different nucleotides when compared with the sequence of the type species *A. psychrotrophicus* (AJ133431). No sequence of β-tubulin or RNA polymerase II of *Antarctomyces* was found in the GenBank. However, we obtained these sequences from *A. pellizariae*, which were then compared with the sequences of the nearest taxa. The β-tubulin sequence analyses placed *A. pellizariae* between the *Thelebolus* (sister genus) (cluster 3) and *Byssochlamys* (cluster 2) groups (Fig. 3b). The RNA polymerase II sequence of *A. pellizariae* (Fig. 3c) formed a separate cluster, but is closed to clusters composed of *Pseudogymnoascus*, *Pseudeurotium*, *Proliferodiscus*, and *Penicillium*.

Antarctomyces pellizariae Menezes, Godinho, Porto, Gonçalves & Rosa, sp. nov.-MB 817786; Figs. 1, 2; Suppl. Fig. 2.

At 22–25 °C, the colonies were 16.5–18 mm in diameter, plane, thin, white with yellowish centre, with irregular margins, anastomosing, reverse uncoloured, with septate hyphae; hyphae 1.5–2 µm broad, thin to thick-walled. Ascomatal initials were scarce and began developing from the coiling of the two side branches, occasionally disposed in tandem. Ascomata were scarce in number, composed of naked asci, single, arising directly from the fertile hyphae, without an exciple. Paraphyses were absent. Asci were 8.5–20 × 6–13 µm in size, subglobose to ellipsoidal, non-stipitate, unitunicate, thick-walled, non-catenate, eight-spored, and developed from croziers. Ascospores were 5–8.5 × 3–6 µm in size (Q = 1.7–1.4), ellipsoidal to fusiform, hyaline, spinulose, thick-walled, without germ pores, one-celled; spines *c*. 0.3–0.5 µm long. Anamorph stage: conidia were absent. Chlamydospores were abundant, 3–5 × 5–10 µm in size (Q = 0.6–0.5), irregular, single or forming long chains, and one or two-celled.

Typus: Antarctica: South Shetland Islands, Robert Island, Coppermine Peninsula, ex snow, 9 Dec

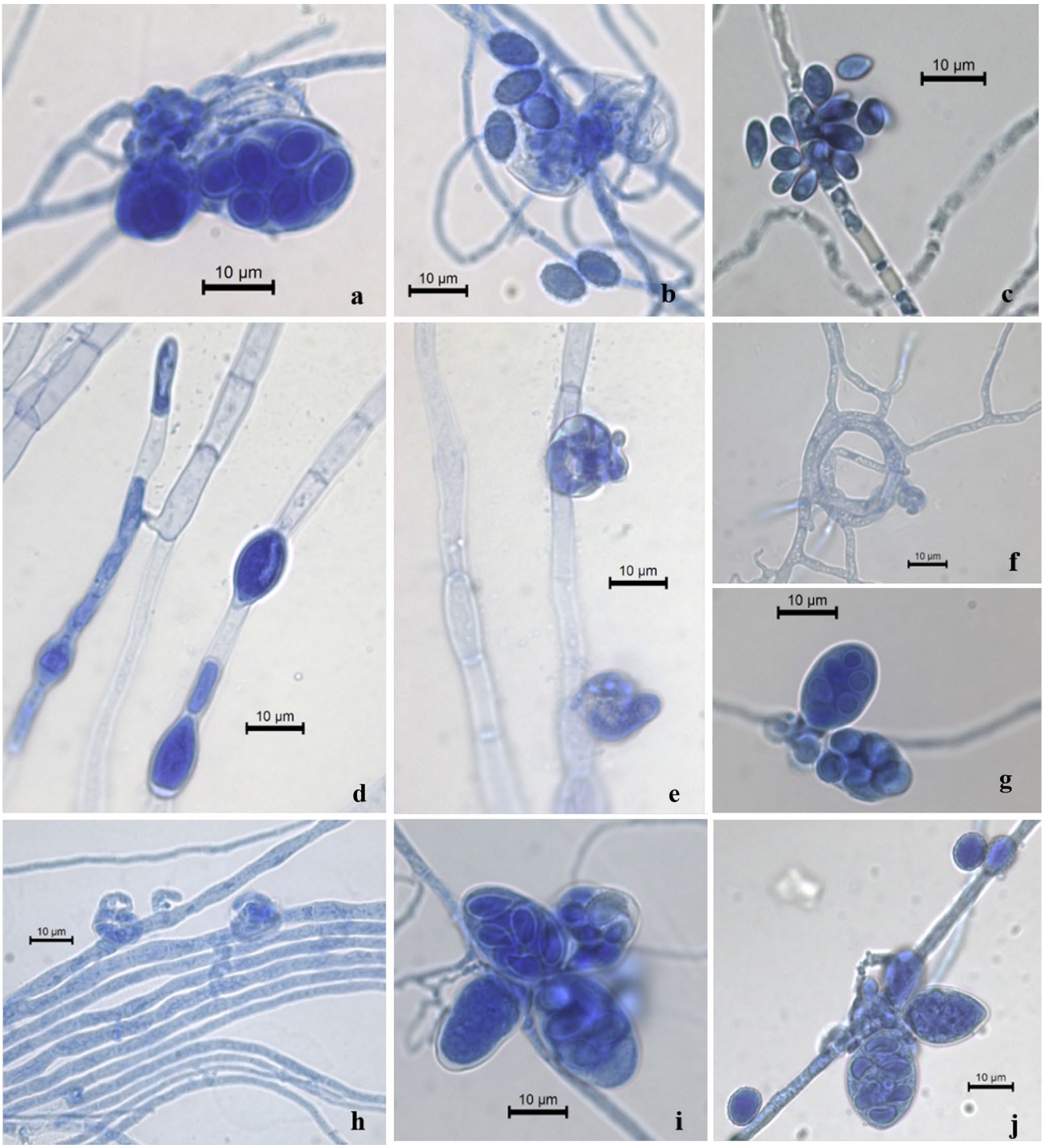


Fig. 3 Phylogenetic analysis of the sequence of *Antarctomyces pellizariae* UFMGCB 12416 compared with sequences of the closest species following BLAST analysis, deposited in the GenBank database. The trees were constructed on the basis of the ITS (**a**), beta-tubulin (**b**), and polymerase II (**c**) regions sequences using the maximum composite likelihood method. T = sequences of type species. STS = sequence of type species. Sequence of *Mucor circinelloides* was used as outgroup (OG)

2015, G. C. A. Menezes, V. N. Gonçalves, V. M. Godinho and L. H. Rosa (UFMGCB 12416—holotype, CBS 142036—ex-type).

Etymology: *Antarctomyces pellizariae* [pe.lli'za.riae. N.L. fem. gen. n. *pellizariae*, of Pellizari, referring to Dr. Vivian Helena Pellizari, a Full Professor of Oceanographic

Institute of University of São Paulo (USP), Brazil, in recognition of her important contributions to microbiology researches in the Brazilian Antarctic Program (PROANTAR), which inspire new Polar Brazilian Microbiologist].

Antarctomyces pellizariae holotypus has been deposited in the Collection of Microorganisms and Cells of Federal University of Minas Gerais (Coleção de Micro-organismos e Células da Universidade Federal de Minas Gerais, UFMG), Belo Horizonte, Minas Gerais, Brazil, as strain UFMGCB 12416, and is permanently preserved in a metabolically inactive state at in cryotubes at −80 °C and in distillate-sterilised water (Castellani 1967) at room temperature. Ex-type culture has been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 142036. The Mycobank number is MB 817786.

Discussion

In the present study, we have described a novel species *A. pellizariae* (Thelebolaceae, Ascomycota) that is endemic to the Antarctic region, after careful consideration of its biological, morphological, physiological, and phylogenetic aspects. The ITS, beta-tubulin, and RNA polymerase II phylogenies showed that *A. pellizariae* is genetically distinct from *A. psychrotrophicus* and other taxa of Thelebolaceae.

The genus *Antarctomyces* Stchigel & Guarro, represented only by the species *A. psychrotrophicus* Stchigel and Guarro, was originally isolated from the soil of South Shetland Islands, King George Island, Antarctica (Stchigel et al. 2001). However, *A. psychrotrophicus* has been recovered from different substrates of Antarctica as a symbiont endophyte of the Antarctic grass *Deschampsia antarctica* (Rosa et al. 2009), in association with macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014), in fresh water lakes (Gonçalves et al. 2012) and thalli of lichens (Santiago et al. 2015). In addition, as *A. psychrotrophicus* has been isolated only from substrates of Antarctic environments, it is considered an endemic psychrophilic species of Thelebolales (Santiago et al. 2015).

The cold biosphere environment characterised by habitats with prolonged cold and freezing temperatures work as a selective ecological filter for all immigrant and resident organisms. These habitats are dominated by cold-adapted (psychrophilic) and cold-tolerant (psychrotolerant) microorganisms (Harding et al. 2011). In contrast with *A. psychrotrophicus*, which has the best growth rate at 25 °C, *A. pellizariae* displayed the best growth at 15 and 20 ± 2 °C and the worst growth at 25 ± 2 °C, indicating that it is indeed a psychrophilic fungus. Usually, psychrophilic microbes possess lipids, enzymes, anti-freezing proteins, and/or carbohydrate-based extracellular polymeric substances, which serve as cryo- and osmo-protectants (Krembs et al. 2011; Stibal et al. 2012). These anti-freezing biological strategies help the microorganisms to support cellular membrane homeostasis as well as the biochemical catalysis (Feller and Gerday 2003). Xiao et al. (2010) characterised *A. psychrotrophicus* as a producer of anti-freeze proteins (Xiao et al. 2010). We have similarly detected that *A. pellizariae* the second described species of the genus, has psychrophilic behavior suggesting that it might serve as a source of anti-freeze compounds. However, further studies are necessary to characterise the possible anti-freeze capabilities of these endemic *Antarctomyces* species.

Conclusion

Antarctic environments present promising habitats to isolate and describe new fungal species. Applying a polyphasic taxonomical approach, we identified a new, endemic, and unique ascomycete named *A. pellizariae*, which represents the second species in the endemic *Antarctomyces*. Since the Antarctic Peninsula has been reported to be one of the main regions of the earth experiencing the effects of global change in climate, species endemic to the Antarctic Peninsula, such as those from the *Antarctomyces* taxa, may provide information about these effects on the resident Antarctic biota. The presence of this unique and endemic fungus, *A. pellizariae* in the Coppermine Peninsula, a specially protected area (SPA) in Antarctica, reinforces the importance of an SPA as a source of new species as well as a biological sanctuary in Antarctica. Finally, *A. pellizariae* displayed psychrophilic behavior and might be a source of interesting anti-freeze compounds that might prove useful in biotechnological processes.

Acknowledgements We acknowledge the financial support from CNPq (Grant No. 407230/2013-0), INCT Criosfera, FAPEMIG, CAPES, and PRPq-UFMG. In addition, we thank the anonymous Reviewer for the values comments and suggestion about the manuscript.

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